```
AGGTCGCAGGCGGGCGTGCGTGGAGCGGGGGCCGCGGCCGCGCCGCAGAGATGTGACTCGGGCCGAAGGC
CAGCTGGAGCGTCGGCGCTGCGGGGCCGCGGGGGTCGAATGTTCGTGGCATCAGAGAGAAAGATGAGAGC
TCACCAGGTGCTCACCTTCCTCCTGCTCTTCGTGATCACCTCGGTGGCCTCTGAAAACGCCAGCACATCC
CGAGGCTGTGGGCTGGACCTCCTCCCTCAGTACGTGTCCCTGTGCGACCTGGACGCCATCTGGGGCATTG
TGGTGGAGGCGGTGGCCGGGGCGGGCGCCCTGATCACACTGCTCCTGATGCTCATCCTCCTGGTGCGGCT
GCCCTTCATCAAGGAGAAGGAGAAGAAGAGCCCTGTGGGCCTCCACTTTCTGTTCCTCCTGGGGACCCTG
GGCCTCTTTGGGCTGACGTTTGCCTTCATCATCCAGGAGGACGAGACCATCTGCTCTGTCCGCCGCTTCC
TCTGGGGCGTCCTCTTTGCGCTCTGCTTCTCCTGCCTGCTGAGCCAGGCATGGCGCGTGCGGAGGCTGGT
GCGGCATGGCACGGGCCCCGCGGGCTGGCAGCTGGTGGGCCTGGCGCTGTGCCTGATGCTGGTGCAAGTC
ATCATCGCTGTGGAGTGGCTGGTGCTCACCGTGCTGCGTGACACAAGGCCAGCCTGCGCCTACGAGCCCA
TGGACTTTGTGATGGCCCTCATCTACGACATGGTACTGCTTGTGGTCACCCTGGGGCTGGCCCTCTTCAC
TCTGTGCGGCAAGTTCAAGAGGTGGAAGCTGAACGGGGCCTTCCTCCTCATCACAGCCTTCCTCTCTGTG
CTCATCTGGGTGGCCTGGATGACCATGTACCTCTTCGGCAATGTCAAGCTGCAGCAGGGGGATGCCTGGA
ACGACCCCACCTTGGCCATCACGCTGGCGGCCAGCGGCTGGGTCTTCGTCATCTTCCACGCCATCCCTGA
GATCCACTGCACCCTTCTGCCAGCCCTGCAGGAGAACACGCCCAACTACTTCGACACGTCGCAGCCCAGG
ATGCGGGAGACGGCCTTCGAGGAGGACGTGCAGCTGCCGCGGGCCTATATGGAGAACAAGGCCTTCTCCA
TGGATGAACACAATGCAGCTCTCCGAACAGCAGGATTTCCCAACGGCAGCTTGGGAAAAAGACCCAGTGG
CAGCTTGGGGAAAAGACCCAGCGCTCCGTTTAGAAGCAACGTGTATCAGCCAACTGAGATGGCCGTCGTG
CTCAACGGTGGGACCATCCCAACTGCTCCGCCAAGTCACACAGGAAGACACCTTTGGTGAAAGACTTTAA
GTTCCAGAGAATCAGAATTTCTCTTACCGATTTGCCTCCCTGGCTGTGTCTTTCTTGAGGGAGAAATCGG
TAACAGTTGCCGAACCAGGCCGCCTCACAGCCAGGAAATTTGGAAATCCTAGCCAAGGGGATTTCGTGTA
AATGTGAACACTGACGAACTGAAAAGCTAACACCGACTGCCCGCCCCTCCCCTGCCACACACACAGACAC
GTAATACCAGACCAACCTCAATCCCCGCAAACTAAAGCAAAGCTAATTGCAAATAGTATTAGGCTCACTG
GAAAATGTGGCTGGGAAGACTGTTTCATCCTCTGGGGGTAGAACAGAACCAAATTCACAGCTGGTGGGCC
AGACTGGTGTTGGTTGGAGGTGGGGGGCTCCCACTCTTATCACCTCTCCCCAGCAAGTGCTGGACCCCAG
GTAGCCTCTTGGAGATGACCGTTGCGTTGAGGACAAATGGGGACTTTGCCACCGGCTTGCCTGGTGGTTT
GCACATTTCAGGGGGGTCAGGAGAGTTAAGGAGGTTGTGGGTGGGATTCCAAGGTGAGGCCCAACTGAAT
CGTGGGGTGAGCTTTATAGCCAGTAGAGGTGGAGGGACCCTGGCATGTGCCAAAGAAGAGGCCCTCTGGG
TGATGAAGTGACCATCACATTTGGAAAGTGATCAACCACTGTTCCTTCTATGGGCTCTTGCTCTAATGT
CTATGGTGAGAACACAGGCCCCGCCCCTTCCCTTGTAGAGCCATAGAAATATTCTGGCTTGGGGCAGCAG
TCCCTTCTTCCCTTGATCATCTCGCCCTGTTCCTACACTTACGGGTGTATCTCCAAATCCTCTCCCAATT
TTATTCCCTTATTCATTTCAAGAGCTCCAATGGGGTCTCCAGCTGAAAGCCCCTCCGGGAGGCAGGTTGG
AAGGCAGGCACCACGGCAGGTTTTCCGCGATGATGTCACCTAGCAGGGCTTCAGGGGTTCCCACTAGGAT
GCAGAGATGACCTCTCGCTGCCTCACAAGCAGTGACACCTCGGGTCCTTTCCGTTGCTATGGTGAAAATT
CCTGGATGGAATGGATCACATGAGGGTTTCTTGTTGCTTTTGGAGGGTGTGGGGGATATTTTGTTTTGGT
TTTTCTGCAGGTTCCATGAAAACAGCCCTTTTCCAAGCCCATTGTTTCTGTCATGGTTTCCATCTGTCCT
GAGCAAGTCATTCCTTTGTTATTTAGCATTTCGAACATCTCGGCCATTCAAAGCCCCCATGTTCTCTGCA
CTGTTTGGCCAGCATAACCTCTAGCATCGATTCAAAGCAGAGTTTTAACCTGACGGCATGGAATGTATAA
ATGAGGGTGGGTCCTTCTGCAGATACTCTAATCACTACATTGCTTTTTCTATAAAACTACCCATAAGCCT
TTAACCTTTAAAGAAAAATGAAAAAGGTTAGTGTTTGGGGGCCGGGGGAGGACTGACCGCTTCATAAGCC
AGTACGTCTGAGCTGAGTATGTTTCAATAAACCTTTTGATATTTCTCAAAAAAAAAAAAAAAAAAAAAAA
(SEQ ID NO:1)
```

FIGURE 1A

MFVASERKMRAHQVLTFLLLFVITSVASENASTSRGCGLDLLPQYVSLCDLDAIWGIVVEAVAG

AGALITLLLMLILLVRLPFIKEKEKKSPVGLHFLFLLGTLGLFGLTFAFIIQEDETICSVRRFL

WGVLFALCFSCLLSQAWRVRRLVRHGTGPAGWQLVGLALCLMLVQVIIAVEWLVLTVLRDTRPA

CAYEPMDFVMALIYDMVLLVVTLGLALFTLCGKFKRWKLNGAFLLITAFLSVLIWVAWMTMYLF

GNVKLQQGDAWNDPTLAITLAASGWVFVIFHAIPEIHCTLLPALQENTPNYFDTSQPRMRETAF

EEDVQLPRAYMENKAFSMDEHNAALRTAGFPNGSLGKRPSGSLGKRPSAPFRSNVYQPTEMAVV

LNGGTIPTAPPSHTGRHLW (SEQ ID NO:2)

FIGURE 1B

underlined = deleted in targeting construct

bold = sequence flanking Neo insert in targeting construct

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AGGTCGCAGGCGGGCGTGCGTGGAGCGGGGGCCGCGGCCGCGCCGCAGAGATGTGACTCG
GGCCGAAGGCCAGCTGGAGCGTCGGCGCTGCGGGGCCGCGGGGGTCGAATGTTCGTGGCA
TCAGAGAGAAAGATGAGAGCTCACCAGGTGCTCACCTTCCTCCTGCTCTTCGTGATCACC
TCGGTGGCCTCTGAAAACGCCAGCACATCCCGAGGCTGTGGGCTGGACCTCCTCCCTCAG
TACGTGTCCCTGTGCGACCTGGACGCCATCTGGGGCATTGTGGTGGAGGCGGTGGCCGGG
GCGGGCGCCCTGATCACACTGCTCCTGATGCTCATCCTCCTGGTGCGGCTGCCCTTCATC
AAGGAGAAGGAGAAGAAGAGCCCTGTGGGCCTCCACTTTCTGTTCCTCCTGGGGACCCTG
GGCCTCTTTGGGCTGACGTTTGCCTTCATCATCCAGGAGGACGAGACCATCTGCTCTGTC
CGCCGCTTCCTCTGGGGCGTCCTCTTTGCGCTCTGCTTCTCCTGCCTGCTGAGCCAGGCA
TGGCGCGTGCGGAGGCTGGTGCGGCATGGCACGGGCCCCGCGGGCTGGCAGCTGGTGGGC
CTGGCGCTGTGCCTGATGCTGGTGCAAGTCATCATCGCTGTGGAGTGGCTGGTGCTCACC
GTGCTGCGTGACACAAGGCCAGCCTGCGCCTACGAGCCCATGGACTTTGTGATGGCCCTC
ATCTACGACATGGTACTGCTTGTGGTCACCCTGGGGCTGGCCCTCTTCACTCTGTGCGGC
AAGTTCAAGAGGTGGAAGCTGAACGGGGCCTTCCTCCTCATCACAGCCTTCCTCTCTGTG
CTCATCTGGGTGGCCTGGATGACCATGTACCTCTTCGGCAATGTCAAGCTGCAGCAGGGG
GATGCCTGGAACGACCCCACCTTGGCCATCACGCTGGCGGCCAGCGGCTGGGTCTTCGTC
ATCTTCCACGCCATCCCTGAGATCCACTGCACCCTTCTGCCAGCCCTGCAGGAGAACACG
CCCAACTACTTCGACACGTCGCAGCCCAGGATGCGGGAGACGGCCTTCGAGGAGGACGTG
CAGCTGCCGCGGGCCTATATGGAGAACAAGGCCTTCTCCATGGATGAACACAATGCAGCT
CTCCGAACAGCAGGATTTCCCAACGGCAGCTTGGGAAAAAGACCCAGTGGCAGCTTGGGG
AAAAGACCCAGCGCTCCGTTTAGAAGCAACGTGTATCAGCCAACTGAGATGGCCGTCGTG
CTCAACGGTGGGACCATCCCAACTGCTCCGCCAAGTCACACAGGAAGACACCTTTGGTGA
AAGACTTTAAGTTCCAGAGAATCAGAATTTCTCTTACCGATTTGCCTCCCTGGCTGTGTC
TTTCTTGAGGGAGAAATCGGTAACAGTTGCCGAACCAGGCCGCCTCACAGCCAGGAAATT
TGGAAATCCTAGCCAAGGGGATTTCGTGTAAATGTGAACACTGACGAACTGAAAAGCTAA
CACCGACTGCCCGCCCCTCCCCTGCCACACACACAGACACGTAATACCAGACCAACCTCA
ATCCCCGCAAACTAAAGCAAAGCTAATTGCAAATAGTATTAGGCTCACTGGAAAATGTGG
CTGGGAAGACTGTTTCATCCTCTGGGGGTAGAACAGAACCAAATTCACAGCTGGTGGGCC
AGACTGGTGTTGGTTGGAGGTGGGGGGCTCCCACTCTTATCACCTCTCCCCAGCAAGTGC
TGGACCCCAGGTAGCCTCTTGGAGATGACCGTTGCGTTGAGGACAAATGGGGACTTTGCC
ACCGGCTTGCCTGGTGGTTTGCACATTTCAGGGGGGTCAGGAGAGTTAAGGAGGTTGTGG
GTGGGATTCCAAGGTGAGGCCCAACTGAATCGTGGGGTGAGCTTTATAGCCAGTAGAGGT
GGAGGGACCCTGGCATGTGCCAAAGAAGAGGCCCTCTGGGTGATGAAGTGACCATCACAT
TTGGAAAGTGATCAACCACTGTTCCTTCTATGGGGCTCTTGCTCTAATGTCTATGGTGAG
AACACAGGCCCCGCCCCTTCCCTTGTAGAGCCATAGAAATATTCTGGCTTGGGGCAGCAG
TCCCTTCTTCCCTTGATCATCTCGCCCTGTTCCTACACTTACGGGTGTATCTCCAAATCC
TCTCCCAATTTTATTCCCTTATTCATTTCAAGAGCTCCAATGGGGTCTCCAGCTGAAAGC
CCCTCCGGGAGGCAGGTTGGAAGGCAGGCACCACGGCAGGTTTTCCGCGATGATGTCACC
TAGCAGGGCTTCAGGGGTTCCCACTAGGATGCAGAGATGACCTCTCGCTGCCTCACAAGC
AGTGACACCTCGGGTCCTTTCCGTTGCTATGGTGAAAATTCCTGGATGGAATGGATCACA
TGAGGGTTTCTTGTTGCTTTTGGAGGGTGTGGGGGATATTTTGTTTTGGTTTTTCTGCAG
GTTCCATGAAAACAGCCCTTTTCCAAGCCCATTGTTTCTGTCATGGTTTCCATCTGTCCT
GAGCAAGTCATTCCTTTGTTATTTAGCATTTCGAACATCTCGGCCATTCAAAGCCCCCAT
GTTCTCTGCACTGTTTGGCCCAGCATAACCTCTAGCATCGATTCAAAGCAGAGTTTTAACC
TGACGGCATGGAATGTATAAATGAGGGTGGGTCCTTCTGCAGATACTCTAATCACTACAT
TGCTTTTTTCTATAAAACTACCCATAAGCCTTTAACCTTTAAAGAAAAATGAAAAAGGTTA
GTGTTTGGGGGCCGGGGGAGGACTGACCGCTTCATAAGCCAGTACGTCTGAGCTGAGTAT
GTTTCAATAAACCTTTTGATATTTCTCAAAAAAAAAAAAAAAAAAAAAAAA
```

FIGURE 2A
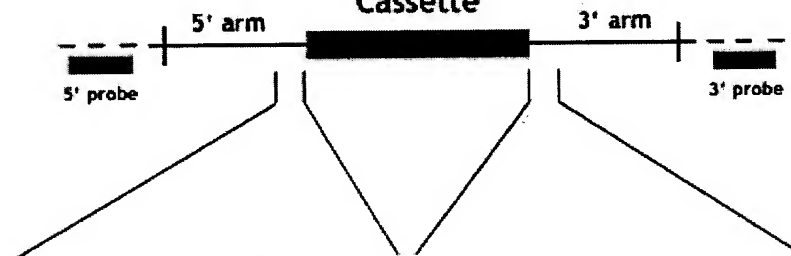
4/5

Gene Sequence
Structure *

526 bp   Sequence Deleted   594 bp

Size of full-length
cDNA: 2870 bp

Targeting Vector*
(genomic sequence)

LacZ-Neo
Cassette

5' arm   3' arm

5' probe   3' probe

Arm Length:
5': 4.2 kb
3': 0.7 kb

———— Targeting Vector
– – – – Endogenous Locus

* Not drawn to scale

5'>TGATGCTCATTCTCCTAGTGA
GACTACCCTTCATCAAGGACAAGG
AAAGGAAGCGGCCTGTGTGCCTCC
ATTTCCTCTTCCTGCTGGGGACCC
TGGGCCTCTTTGGCCTGACGTTTG
CCTTCATCATCCAGATGGACGAGA
CAATCTGCTCCATCCGACGCTTCC
TCTGGGGTGTCCTCTTCGCGCTCT
GCTTTTCCGCT<3' (SEQ ID
NO:3)

5'>GTGAGCCTGGCACTGTGCCTG
ATGCTGGTGCAGGTCATCATTGCC
ACTGAGTGGCTGGTGCTGACTGTG
CTGCGTGACACGAAGCCAGCCTGC
GCCTACGAGCCCATGGATTTTGTG
ATGGCGCTCATCTACGACATGGTG
CTGCTGGCCATCACCCTGGCCCAG
TCCCTCTTCACGCTGTGTGTGGCAAG
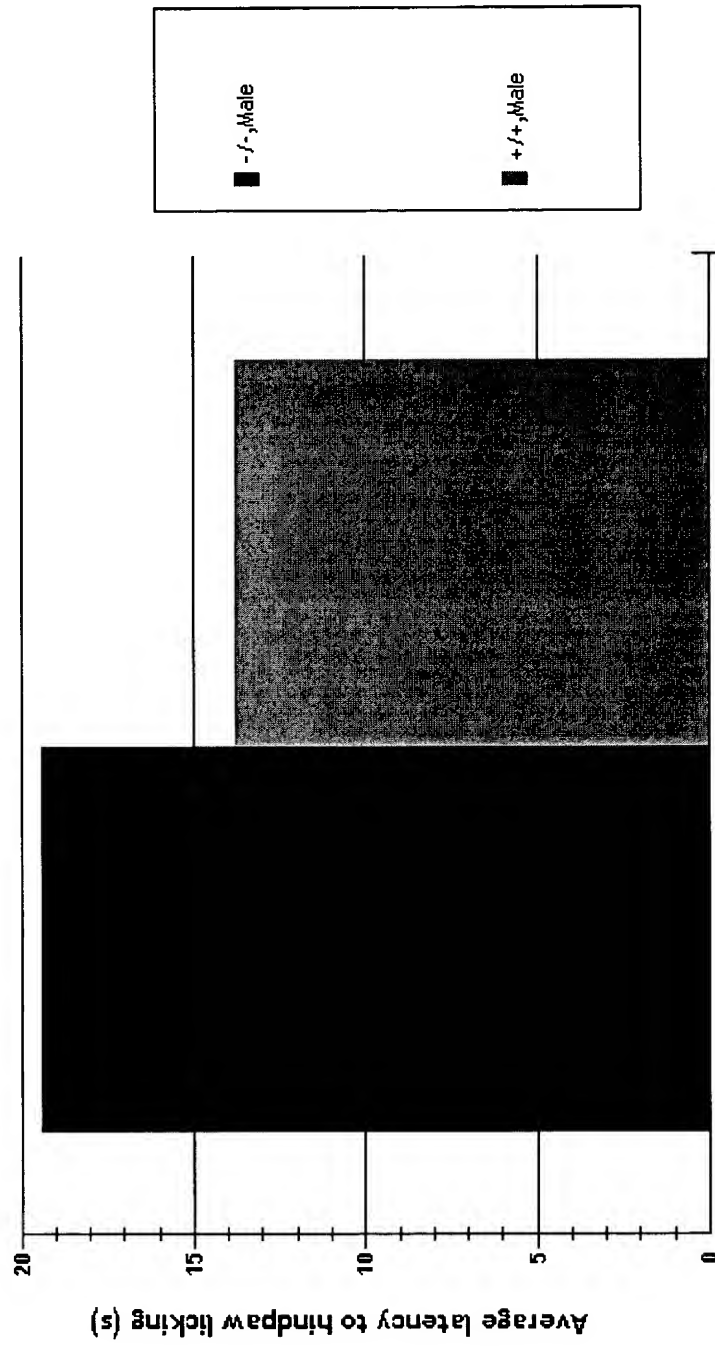TTCAAACGGTG<3' (SEQ ID
NO:4)

FIGURE 2B

Hot Plate Test

Average latency to hindpaw licking (s)

20 15 10 5 0

- -/-,Male
- +/+,Male

FIGURE 3